REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111

Serial Number: 09/458,862

Filing Date: December 10, 1999

Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

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In the Claims

Please amend the claims as follows:

- 1. (Currently Amended) A cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, a cryoprotective agent that penetrates the cell membrane, which agent is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.
- 2. (Original) The cryopreservation medium of claim 1 wherein the cells are peripheral blood lymphocytes.
- 3. (Original) The cryopreservation medium of claim 1 that comprises arabinogalactan.
- 4. (Canceled)
- 5. (Currently Amended) The cryopreservation medium of claim 4 <u>1</u> wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 6. (Original) The cryopreservation medium of claim 1 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
- 7. (Original) The cryopreservation medium of claim 1 which does not comprise protein.
- 8. (Original) The cryopreservation medium of claim 1 which is infusible.

9-10. (Canceled)

- 11. (Original) The cryopreservation medium of claim 1 wherein the cells are human cells.
- 12. (Original) The cryopreservation medium of claim 1 wherein the cells are non-human vertebrate cells.
- 13. (Canceled)
- 14. (Currently Amended) A composition suitable for administration to a human, comprising a suspension of cells in a cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, wherein arabinogalactan, or a biological or functional equivalent thereof, is present in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof,

and wherein the medium does not comprise dimethylsulfoxide or serum.

- 15. (Canceled)
- 16. (Previously Presented) The composition of claim 14 wherein the cells are peripheral blood lymphocytes.
- 17. (Currently Amended) The composition of claim 14 wherein at least one of the eryoprotective agents is the medium comprises arabinogalactan.
- 18. (Canceled)

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- 19. (Previously Presented) The composition of claim 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 20. (Previously Presented) The composition of claim 14 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
- 21. (Previously Presented) The composition of claim 14 which does not comprise protein.
- 22. (Previously Presented) The composition of claim 14 which is infusible.
- 23. (Canceled)
- 24. (Previously Presented) The composition of claim 14 wherein the cells are human cells.
- 25. (Canceled)
- 26. (Currently Amended) A method for preserving cells comprising:
- electrolyte solution, and at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*; and
 - (b) freezing the cell suspension to yield a frozen cell suspension.

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- 27. (Original) The method of claim 26 further comprising thawing the frozen cell suspension under conditions that maintain cell viability.
- 28. (Original) The method of claim 26 wherein the cells are human cells.
- 29. (Canceled)
- 30. (Original) The method of claim 26 wherein the cells are peripheral blood lymphocytes.
- 31. (Currently Amended) A frozen composition comprising i) <u>inorganic salts capable of maintaining physiological pH when in solution a balanced electrolyte solution</u>, ii) at least one eryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, <u>iii) a cryoprotective agent that penetrates the cell membrane</u>, and <u>iii) iv)</u> freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum.
- 32. (Original) A frozen hematopoietic cell-containing composition made according to the method of claim 26.
- 33. (Original) The cryopreservation medium of claim 5 wherein the cryoprotective agent that penetrates the cell membrane is glycerol.
- 34. (Original) The cryopreservation medium of claim 33 wherein the concentration of glycerol is about 1% to about 3%.
- 35. (Previously Presented) The cryopreservation medium of claim 1 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

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36. (Previously Presented) The composition of claim 14 or 31 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

- 37. (Previously Presented) A cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the balanced electrolyte solution is selected from the group consisting of lactated Ringer's solution, PlasmaLyte-ATM, Normosol-RTM, Veen-DTM, Polysal®, and Hank's balanced salt solution.
- 38. (Previously Presented) The cryopreservation medium of claim 37 wherein the lymphocytes are peripheral blood lymphocytes.
- 39. (Previously Presented) The cryopreservation medium of claim 37 wherein the agent is arabinogalactan.
- 40. (Previously Presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent that penetrates the cell membrane.
- 41. (Previously Presented) The cryopreservation medium of claim 40 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 42. (Previously Presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
- 43. (Previously Presented) The cryopreservation medium of claim 37 which does not comprise protein.

- 44. (Previously Presented) The cryopreservation medium of claim 37 which is infusible.
- 45-46. (Canceled)
- 47. (Previously Presented) The cryopreservation medium of claim 37 wherein the cells are human cells.
- 48. (Previously Presented) The cryopreservation medium of claim 37 wherein the cells are non-human vertebrate cells.
- 49. (Previously Presented) The method of claim 26 wherein the medium comprises arabinogalactan.
- 50. (Canceled)
- 51. (Currently Amended) The method of claim 50 26, 57 or 58 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 52. (Currently Amended) The method of claim 26, 57 or 58 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.
- (Currently Amended) A cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, a cryoprotective agent that penetrates the cell membrane, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.

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- (Currently Amended) A cryopreservation medium comprising a balanced electrolyte 54. solution, incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.
- (Currently Amended) A frozen composition comprising i) inorganic salts capable of 55. maintaining physiological pH when in solution a balanced electrolyte solution, ii) at least one ervoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) a cryoprotective agent that penetrates the cell membrane, and-iii) iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.
- (Currently Amended) A frozen composition comprising i) inorganic salts capable of 56. maintaining physiological pH when in solution a balanced electrolyte solution, ii) at least one eryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.
- (Currently Amended) A method for preserving cells comprising: freezing a cell 57. suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to

about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

- 58. (Currently Amended) A method for preserving cells comprising:
- (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution, and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum; and
- (b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.
- 59. (New) The medium of claim 1, 37, 53 or 54 wherein the post-thaw survival rate is at least about 40%.
- 60. (New) The method of claim 26, 57 or 58 wherein the post-thaw survival rate is at least about 40%.
- 61. (New) A cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane.
- 62. (New) The medium of claim 61 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycerol.
- 63. (New) The medium of claim 62 wherein glycerol is about 1% to about 5%.

(New) The cryopreservation medium of claim 61 wherein the arabinogalactan is about 64. 10% w/v to about 30% w/v.